

THE MARINE SAND-DWELLING CILIATES OF CAPE COD

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MARINE SANDS AND PSAMMOPHILOUS CILIATES

It is well known that the marine sands between the tidemarks, or deeper along the seashore, constitute some quite peculiar and interesting biotopes. With regard to their fauna, one can distinguish the sand-boring animals of large or small size and, on the other hand, the very minute ones which live in the intergranular spaces of the sand and constitute an "interstitial" microfauna.

This interstitial microfauna, or mesopsammon, includes, besides numerous copepods, nematodes, gastrotrichs, etc., many protozoa and most particularly numerous ciliates. Kahl (1933, 1935) has recorded more than 80 ciliate species, belonging to about 20 different families, from the sandy bottoms of Kiel and Helgoland.

I found many of these infusorian species and some new ones in the sand of the tidal zone of different beaches in Brittany (Fauré-Fremiet, 1950). This result ties up with some other dispersed data and agrees with a general statement of Calkins (1933) who emphasizes the cosmopolitan distribution of the marine littoral protozoan species. But, from an ecological point of view, it can be noted that the size of the intergranular spaces acts as a selective factor in the distribution of the interstitial ciliates.¹

The intergranular spaces may be roughly estimated from the dimensions of the solid grains which bound them; a comparison of the granulometric curves plotted for different kinds of sand with the associated interstitial fauna leads one to distinguish in the fauna two different types of ciliates.

If the mean size of the sand grains exceeds 0.4 mm., the interstitial ciliate fauna includes some characteristic sand-living species and some occasionally sand-dwelling infusorians which are much more numerous. The characteristic sand-living species are generally characterized by their small or medium size, their flattened shape, and their jerky motion, with alternately very fast swimming and sudden stopping when in contact with solid surfaces. The occasional sand-dwellers, quite varied in shape and size and usually widespread along the seashore, display a common but more or less accentuated thigmotactic behavior. All these together constitute, in the medium and coarse sands, what I have called the mesoporal infusorian fauna (Fauré-Fremiet, 1950).

If the mean size of the sand grains falls between 0.1 and 0.3 mm., their inter-spaces shelter some very peculiar species of ciliates, generally thread-like or ribbon-like, often as long as one or two millimeters, always strongly thigmotactic. These

¹ In a previous paper (Fauré-Fremiet, 1950) are discussed some of the physical and chemical conditions which depend on the enormous development of the solid-liquid interfaces of the sand; they probably play an important role in interstitial life of the sand-dwelling ciliates.

characteristic sand-dwelling species constitute what I have called the microporal infusorian fauna.

It must be said, however, that some ciliates of the microporal type can live as well in the coarse as in the fine sand, and these species may be called euryportal.

Finally, if the mean size of the sand grains is below 0.1 mm., or if a coarser sand is so muddy that the intergranular spaces are filled with very minute organic particles, the porosity becomes incompatible with the interstitial life of any species of ciliates.

Numerous observations made on different beaches and sandy flats of the Cape Cod region during the months of June and July, 1950, offer new evidence of the cosmopolitan distribution of many psammophilous ciliate species, and of the general significance of the two types of infusorian interstitial fauna.

TECHNIQUE

The superficial layers of wet sand exposed at low tide were collected with a spoon, to a depth of 2 to 10 millimeters, and put in some convenient bottle or carton, with just a little sea water. At the laboratory, the samples were transferred to large fingerbowls, covered with sea water and allowed to stand overnight.

In order to observe the ciliates, a small quantity of sand, taken from one sample with a spatula or a pipette, was put in a Petri dish and gently agitated with sea water to which a few drops of a 12 per cent $MgCl_2$ solution were added.²

Then the sand was gathered at one side of the dish and the water examined under a low-power binocular microscope. The psammophilous ciliates, now swimming or gliding on the glass bottom of the dish, are easily picked up with a capillary pipette and transferred to a slide for a more careful examination, or placed in small covered dishes with normal sea water and sand grains for later examination.

The granulometric analyses of three different types of sand were made at the Woods Hole Oceanographic Institution. The corresponding curves were plotted for the very fine sand of the Barnstable Harbor flats, and for the two coarse sands from Tarpaulin Cove and Nobska beaches (Fig. 1).

A rough micrometric estimation of the mean diameter of the grains was quite sufficient for the comparative study of many other samples.

MESOPORAL INFUSORIAN FAUNA

The sands of almost all the beaches around Woods Hole shelter ciliate species of the mesoporal type.

The samples examined were collected in several places on different beaches at Penzance, Woods Hole (M. B. L. beach), Sippiwisset, Quisset, Gansett and Nona-messet along the Buzzards Bay coast, and at Tarpaulin Cove, Nobska, Falmouth, and Great Pond along the Vineyard Sound.

In every case the granulometric measurements show that the size of most of the sand grains lies roughly between 0.3 mm. and 1.0 mm. These sands are quartzous, with very few mica flakes, and contain, with few or no mud particles, a variable amount of organic residues.

² See Fauré-Fremiet (1950). Magnesium chloride, which narcotizes many small metazoans rapidly, decreases the thigmotactic adherence of the ciliates, and hence releases them from the sand particles, without a sensible alteration of their motility.

In these somewhat coarse sands, the ciliates are generally neither abundant nor very varied; they belong most often to diatom-ingesting species.

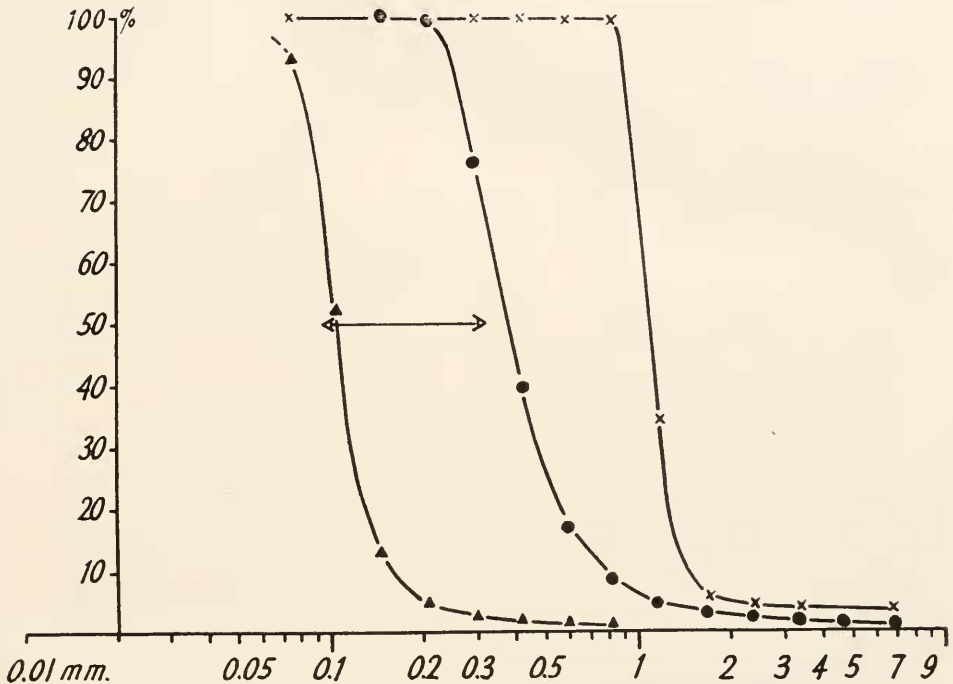


FIGURE 1. Granulometric characteristics of sands collected on three different beaches of Cape Cod. Cumulative curves; abscissa: diameter, in millimeters, of the sand grains (logarithmic scale); ordinate: weight per cent of the sand grains larger than the corresponding diameter. $\blacktriangle$ Barnstable Harbor flat; $\bullet$ Tarpaulin Cove; $\times$ Nobska beach.

The typically mesoporal species observed are the following:

Holotricha Gymnostomata:

Mesodinium pulex, f. *pupula* Kahl

Placus (*Thoracophyra*) *buddenbrocki* Sauerbrey

Remanella margaritifera (?) Kahl

Oligotricha:

Strombidium sauerbreyae Kahl

Hypotricha:

Discocephalus rotatorius Ehrb.

The other species, widely distributed along the seashore, belong occasionally, but not specifically, to the mesoporal fauna; the following are frequently encountered:

Holotricha Gymnostomata :

Lacrymaria sp.
Coleps tessellatus

Holotricha Hymenostomata :

Pleuronema sp.
Frontonia sp.

Heterotricha :

Condylostoma patens (O.F.M.) Stein

Hypotricha :

Epiclintes ambiguus O.F.M.
Euplotes sp.
Diophrys scutum Duj.
Uronychia transfuga O.F.M.
Aspidisca major Madsen

Finally, some sand-living ciliates of euryportal character are often present :

Holotricha Gymnostomata :

Trachelocerca phaenicopterus
Trachelocerca sp.

Hypotricha :

Holosticha fasciola (?) Kahl

It is apparent that these different vegetarian species seem to be living quite independently of one another. Their quantitative distribution appears quite variable and unequal, not only from one beach to another but also along the same beach, between two areas only a few meters distant and showing the same physical appearance. Sometimes a local population of one of these species may be fairly abundant, probably in connection with the local growth of some species of diatom or bacteria utilized as food. This local growth is conditioned itself, perhaps, by some slight local difference in the intensity of light or the tidal agitation.

MICROPORAL INFUSORIAN FAUNA

Some of the special conditions needed by the ciliates of the microporal type are found in the sand of the Barnstable Harbor flats, on the south coast of Cape Cod Bay. There the sand is very fine, slightly muddy, closely packed, and inhabited by many burrowing invertebrates, such as clams and worms.

The ciliates seem to be confined to the superficial layer of clear gray sand, sometimes reddish spotted by purple bacteria, and covering a deeper black sand rich in organic decomposition products.

The size of the sand grains varies from 0.05 mm. to 0.2 mm. In many samples collected on the flats exposed at low tide, there was found a more or less abundant and varied association of the following characteristic microporal species :

Holotricha Gymnostomata :

Helicoprordon (Chaenea) gigas Kahl

Centrophorella fasciolata Sauerbrey

Centrophorella lanceolata n. sp.

Geleia simplex n. sp.

Geleia decolor Kahl

Geleia fossata Kahl

Geleia orbis Fauré-Fremiet

Remanella sp.

Heterotricha :

Condylostoma remanei Spiegel, Kahl

Blepharisma clarissimum Anigstein

Blepharisma sp.?

Hypotricha :

Urostrongylum caudatum Kahl

Besides this very specific microporal fauna of ciliates, one observes some of the euryportal species already mentioned, and the very curious *Parablepharisma peltatum* Kahl; this is much more abundant in a different biotope, such as the felting of a dense algal littoral flora in some brackish-water ponds.

It can be noted that on the Barnstable flats, as well as on the Brittany beaches, the different species of *Geleia* behave like saprobic ciliates; they are fairly abundant where purple bacteria grow. On the other hand, different species of *Centrophorella*, *Remanella* and *Blepharisma* are commonly encountered in the same biotope with *Geleia*, and they may also be found in clean fine sand and occasionally in the somewhat coarse sand of the open beaches, with some of the characteristic species of the mesoporal infusorian fauna.

COMPARATIVE STUDY OF THE CILIATE INTERSTITIAL FAUNA

According to the results above, the mesoporal and the microporal infusorian fauna show respectively the same characteristics in the littoral sands of Cape Cod and in those of Brittany. However, the mean size of the sand grains available for each of the two different interstitial faunas seems to be slightly lower on Cape Cod than on the Brittany coasts; this may be due to the presence in Brittany beaches of numerous mica flakes which assure a more compact packing of the sand grains than the almost pure quartzous particles of the Cape Cod sands. In other words, with equal diameter of sand grains, the interspaces are larger between angular particles than between flat and angular particles mixed together; it is known that the dimensions of these spaces act as an important ecological factor in the distribution of ciliates (Fauré-Fremiet, 1950).

As a matter of fact, among the different Cape Cod sandy shores examined, the typical microporal infusorian fauna was only encountered in sands of the Barnstable Harbor flats. Very fine, slightly muddy, filled with numerous sand-boring animals such as annelids and clams, rich in organic residues and in sulfur bacteria, this sand resembles closely in its microfauna the sand of one beach studied in

Brittany, namely, the beach of Moustierlin, near Concarneau (Fauré-Fremiet, 1950). However, Barnstable Harbor shows some estuarine characteristics, such as some tidal variations of the sea water salinity.

Considering the cosmopolitan character of the marine littoral ciliates, the data gained from study of the Cape Cod sandy shores need some discussion. Many of the ciliates found there can be identified without hesitation, and belong to species already described, and found in the European beaches or sandy grounds. Some others appear very closely related to known species. Finally, some seem to be new species.

The following species in the sands of the Cape Cod region were positively identified with those described in the Brittany coast (Fauré-Fremiet, 1950) and the Bay of Kiel (Kahl, 1933, 1935) :

Holotricha Gymnostomata :

- Mesodinium pulex* f. *pupula* Kahl
- Placus* (*Thauracophrya*) *buddenbrocki* Sauerbrey
- Helicoprorodon* (*Chaenea*) *gigas* Kahl (Fauré-Fremiet, 1950)
- Centrophorella fasciolata* Sauerbrey, Kahl
- Coleps tessellatus* Kahl

Heterotricha :

- Condylostoma patens* O.F.M.
- Condylostoma remanci* Spiegel
- Blepharisma clarissimum* Anigstein
- Parablepharisma pellitum* Kahl

Hypotricha :

- Epiclintes ambiguus* O.F.M.
- Urostrongylus caudatus* Kahl
- Discocephalus rotatorius* Ehrb.
- Diophrys scutum* Duj.
- Urorychia transfuga* O.F.M.

Among these ciliates, *Discocephalus rotatorius* requires special mention; this apparently uncommon infusorian, first described by Ehrenberg from the seashore of the Sinai peninsula, was again observed by Sauerbrey (1928) on the sand grounds of the Bay of Kiel. The form found in the coarse sand of Tarpaulin Cove shows not only the same morphological characteristics, but also the same physiological peculiarity reported by Sauerbrey, namely, the ingestion of small sand particles.

Some species, such as *Condylostoma patens*, show great variability of shape and size, but not of structure; however, this does not make identification difficult. On the other hand, it is hard to decide for some other species whether the differences observed between populations living in different regions are of specific value, or merely define some geographic varieties, or result from poor descriptions in the literature.

Among the Holotricha, *Gelcia orbis*, very abundant in the sand of Barnstable Harbor, seems to be more flattened and more thigmotactic than the Brittany form

(Fauré-Fremiet, 1950), but one does not observe any significant differences between these, either as to size (up to 1.5 mm. in full extension) or structure.

Geleia decolor Kahl is apparently identical with the Brittany species which I described under this name, but both are slightly smaller than the Kahl species and differ perhaps from his by the presence of a little anterior and preoral pit.

Among the Heterotricha a species of *Blepharisma* with a beaded macronucleus, very common in Barnstable Harbor, should be considered as *B. clarissimum* forma *arenicola* Kahl; it is smaller than the latter form, however, being 275 to 300 micra as opposed to 450 micra (according to Kahl). It seems to be similar to a form observed by Dragesco (personal communication) in the sand at Roscoff. On the other hand, *B. clarissimum* Anigstein is so well characterized by its cytoplasm (refrangent, transparent and hyaline, like that of an *Opalina*) that the so-called "forma arenicola" must be considered as a distinct species.

Among the Hypotricha, a microporal Holosticha with numerous macronuclear beads is shaped nearly like *H. extensa* Kahl, with two frontal cirri; it is a little larger than Kahl's species (about 300 micra *contra* 140 to 240 micra) and possesses four or five transverse cirri like *H. fasciola* Kahl, as against 6 to 7 as in *H. extensa*. But the latter species is narrower and possesses three frontal cirri at least.

A mesoporal Aspidisca, measuring about 50 to 55 micra, resembles very closely *A. major* Madsen, in its two elongated macronuclear pieces and its five transverse cirri (as opposed to 6 for *A. binucleata* Kahl, and 11 to 12 for *A. polystyla* Stein). However, the Madsen species is larger: 60 to 90 micra.

Three presumably new species need now a more detailed description.

NEW SPECIES

Centrophorella lanceolata n. sp. (Fig. 2)

The enigmatic genus *Centrophorella* (see Kahl, 1933, 1935 and Fauré-Fremiet, 1950), first described by Sauerbrey under the name *Kentrophoros*, probably belongs to the family Amphileptidae, according to Kahl.

The different species are strongly thigmotactic, very fragile and difficult to examine *in vitro*. The use of $MgCl_2$ solution shows that they are much more frequently represented in the microporal interstitial fauna than it was supposed at first. I did not observe in the fine sand of Barnstable Harbor the very large species *C. fistulosa* Fauré-Fremiet of the Brittany coast, but I found, besides *C. fasciolata* Sauerbrey, a new one which I call *C. lanceolata* n. sp. It seems highly probable that it can be found elsewhere, and a form recently observed at Roscoff, in Brittany, by Dragesco (personal communication) is perhaps the same.

Centrophorella lanceolata is a very flattened and ribbon-like ciliate, measuring about 460 to 520 micra in length, 40 micra wide and 5 to 6 micra thick. It tapers at the ends; the anterior part is slender, with a sort of neck preceding the buccal edge, curved on the right side and bearing a row of long cilia. The posterior end is pointed.

The ventral surface is ciliated, and the dorsal one is covered with an accumulation of dark sulfur bacteria, except at a narrow lateral margin at the neck and at the tail.

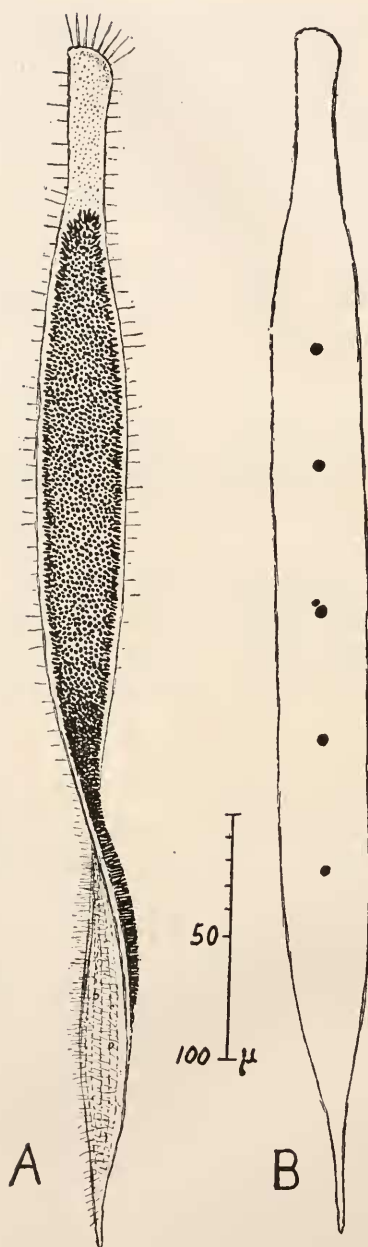


FIGURE 2. *Centrophorella lanceolata* n. sp. A: View of the living infusorian, showing the dorsal brush of sulfur bacteria. B: Outline of the ciliate showing, after staining, the size and the position of five macronuclei and one (probably) micronucleus.

The associated sulfur bacteria, rod-shaped and all of the same length, measure about 10 micra; they are attached to the dorsal surface of the ciliate infusorian body by one end, and arranged in a parallel manner like the bristles of a brush. That is a characteristic of the so-called Caulobacteria—the genus *Pasteuria* (Metschnikoff), for example. As is the case among others of this group, they divide longitudinally.

The dark appearance of the bacteria of *C. lanceolata* is due to the presence in their cytoplasm of highly refractile sulfur granules; in fact, these bacteria are really colorless, as are those of *C. fistulosa*, whereas those of *C. fasciolata* show a pink color.

It is known that the nuclei of the *Centrophorella* (Kahl, 1933, 1935; Fauré-Fremiet, 1950) are very small; *C. lanceolata* shows five to six globular macronuclei about 4 micra in diameter, and some smaller micronuclei.

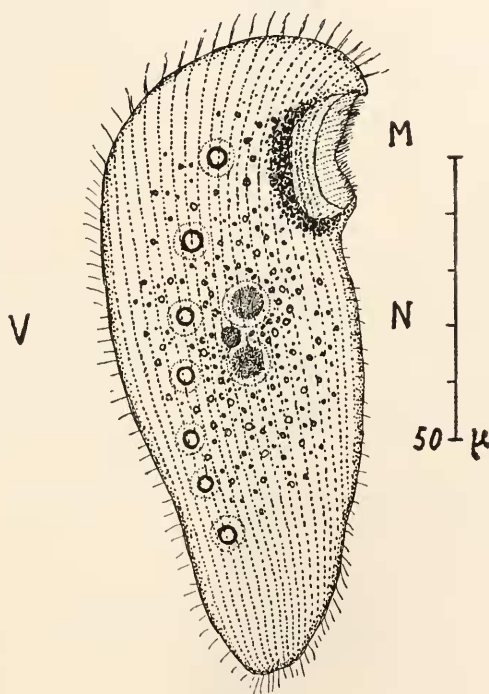


FIGURE 3. *Remanella obtusa* n. sp. View of the ciliated right side. M, mouth; N, double macronucleus with one micronucleus; V, Müller's vesicles with refringent bodies.

The association of the *Centrophorella* with the Caulobacteria is a perfectly constant character; in each case these bacteria are sulfur bacteria, but they differ in size and color with each species of *Centrophorella*. It would be interesting to define the nature, specific or adaptively induced, of these differences. On the other hand, it is noteworthy that the bacteria constantly associated with a heterotrichous ciliate, *Parablepharisma pellitum* Kahl, are quite different and do not contain sulfur.

Remanella obtusa n. sp. (Fig. 3)

Whereas the different known species of *Remanella* belong to the microporal interstitial fauna, this new one was found many times in the relatively coarse sand of Nobska beach.

At first sight, *R. obtusa* recalls some species of *Chlamydodon* by its shape and its thigmotactic behavior. This ciliate is very flat, yellowish in color, with a brown pigmented area around the mouth. It measures about 110 micra in length and is 40 micra wide. Its anterior edge is broadly rounded.

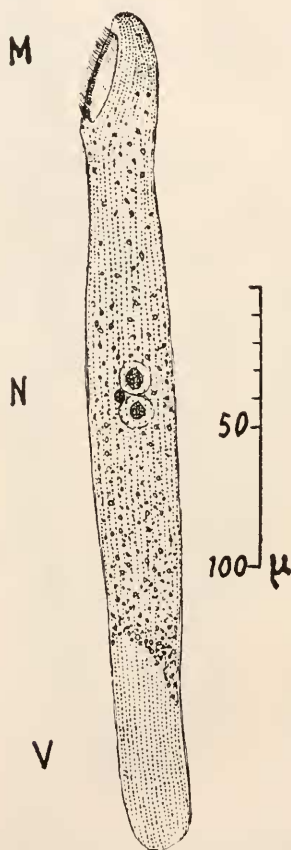


FIGURE 4. *Gelcia simplex* n. sp. M, mouth; N, double macronucleus with one micronucleus; V, large posterior contractile vesicle.

The mouth appears as a lateral semi-circular notch without pharyngeal prolongation. The Müller's vesicles, with a large refringent granule in each one, are 6 to 8 in number. The animal possesses two macronuclei, each containing a central chromatic body, and a single micronucleus.

Remanella obtusa is ciliated only on its flat right side; it is very thigmotactic, and glides rapidly on the surface of the sand grains. It is a diatom feeder.

Geleia simplex n. sp. (Fig. 4)

Geleia simplex, frequently encountered in the fine sand of the Barnstable Harbor flats, differs from the other species of the genus (see Kahl, 1933, 1935; Fauré-Fremiet, 1950) by the lack of an anterior prebuccal lobe, the nearly apical location of the mouth, and the presence of a large posterior vacuole. Its length is about 260 to 300 micra, and its width about 30 micra. Its flexible and slightly contractile body is more or less flattened.

G. simplex possesses two macronuclei with large karyosomes, and one micronucleus.

The mouth is an oval depression extending anteriorly between two body rows of cilia. The buccal ciliation includes a fringe of large cilia on the right side and on the left one a field of fine undulating cilia.

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SUMMARY

1. Two categories of ciliate species dwell in the damp sand of marine beaches; some of these seem to be so closely adapted to interstitial conditions that they do not live outside; some others enter the sand readily and can live in it as well as anywhere else along the seashore, where they are ordinarily widespread.

2. With regard to the size of the interstitial sand spaces, which are roughly proportional to the size of the grains, the sand-dwelling ciliates can be divided into two ecological classes:

In coarse sands, with a mean diameter of the grains above 0.4 mm., the mesoporal infusorian fauna includes a few species exclusively sand-living and many occasional sand-dwelling forms.

In fine sands, with a mean grain diameter of 0.3 to 0.7 mm., the microporal infusorian fauna is composed of true sand-living ciliate species.

In addition, some typical interstitial species, widespread in fine and coarse sands, are called euryportal.

3. Comparison of the ciliate interstitial fauna observed in Brittany and Cape Cod beaches shows that interstitial species are cosmopolitan, and that the distinguishing characteristics of mesoporal and microporal fauna have a generalized ecological significance. On the other hand, it appears that the truly microporal species are endowed with some peculiarities very interesting from the standpoint of cytology, cell-physiology and micro-ecology.

LITERATURE CITED

- CALKINS, G. N., 1933. The biology of the protozoa. Lea and Febiger, Philadelphia. (2nd Edition.)

- FAURÉ-FREMIET, E., 1950. Ecologie des Ciliés psammophiles littoraux. *Bull. Biol. de la France et de la Belgique*, **84**: 35-75.
- KAHL, A., 1933. Ciliata libera et ectocommensalia. In: Grimpe und Wagler, Thierwelt der Nord- und Ostsee. Akad. Verl., Leipzig.
- KAHL, A., 1935. Wimpptiere oder Ciliata. In: Fr. Dahl, Die Tierwelt Deutschlands. Fischer, Jena.
- SAUERBREY, E., 1928. Beobachtungen über einige neue oder wenig bekannte marine Ciliaten. *Arch. f. Protistenk.*, **62**: 355-407.
- SPIEGEL, A., 1926. Einige neue marine Ciliaten. *Arch. f. Protistenk.*, **55**: 184-190.